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It is herewith certified by the Austrian Patent Office that

Frank MATTNER

at A-1180 Vienna, Krottenbachstraße 267

on September 12, 2003 filed a patent application relating to

"apheresis device"

and that the specification herewith annexed is identical with the specification as originally filed together with this patent application.

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VERIFICATION

I, Kerstin Kiss, of Riemergasse 14, A-1010 Vienna, Austria, do hereby declare

that I am conversant with the German and English languages and that to the best of

my knowledge and belief the following is a true and correct translation made by me of

the accompanying Official Copy of the Austrian Patent Office relating to

Patent Application No. A 1444/2003 filed on 12 September 2003.

Signed this 25th day of March 2009

Kerstin Kiss

Apheresis Device

The invention relates to an apheresis device comprising a solid carrier capable of being contacted with the blood or plasma flow.

By apheresis, treatment methods are to be understood whose therapeutic effects are based on the extra-corporeal elimination of pathogenic proteins, protein-bound pathogenic substances, free pathogenic substances or pathogenic cells of the blood. If the pathogenic protein can only be eliminated from the cell-free plasma, the plasma previously is separated from the blood cells by means of a membrane plasma separator (plasma separation) or by means of a haemocentrifuge. In the non-selective plasma exchange (plasmapheresis), the exchanged patient plasma is separated as a whole, wherein besides the pathogens, also all other vital proteins are eliminated. On account of this, substitution of the removed plasma with electrolytes, human albumin or fresh plasma is necessary. In selective plasmapheresis methods, pathogenic proteins can quite specifically be removed from the separated plasma with the help of adsorption, precipitation or filtration, it being possible to re-infuse the plasma without a substantial loss of volume after the removal has been effected. These selective methods have the advantage that one can do without a substitution solution. In selective whole blood apheresis methods, the pathogenic proteins are specifically adsorbed directly from the non-pretreated blood without a previous plasma separation, whereby - in contrast to the plasma separation methods - both the plasma separation and the addition of a substitution solution can be omitted. A further sub-form of apheresis is cytapheresis, in which cells are removed from the blood. With this, leucocytes, erythrocytes, thrombocytes, granulocytes or even stem cells can be recovered selectively.

Even though apheresis (e.g. as plasmapheresis or cytapheresis) currently is mainly used for the recovery of donor plasma (as a plasma pack, for the isolation of various plasma fractions or for the recovery of blood products) apharesis methods are becoming increasingly important in the field of therapy. Currently, a whole series of metabolic illnesses (e.g. (familial) hypercholesterinemia, progressive coronary heart disease with isolated Lp(a) increase, chylomicronemia syndrome, liver fail-

ure, ...), renal diseases (Goodpasture Syndrome, systemic Lupus erythematodes with lupus nephritis, Wegener's granulomatosis, hemolytic-uremic syndrome, idopathic focal-sclerosing glomer-ulonephritis, paraproteinemia-associated syndromes, cryoglobu-lonemic purpura, HLA sensitization in case of kidney transplantation, ...) diseases of the nervous system (Myastenia gravis, Guillain-Barré syndrome, chronic demyelinizing polyra-diculoneuritis, paraproteinemic polyneuropathy, Lambert-Eaton syndrome, Refsum syndrome, ...), diseases of the immune system (rheumatoid arthritis, immune inhibitor hemophilia, pemphigus,...), diseases of the circulatory system and of micro-circulation (hyperviscosity syndrome, antiphospholipid antibody syndrome, thrombotic microangiopathy after bone marrow trans-plantations, age-related macular degeneration, acute hearing loss, peripheral disturbances of microcirculation, idiophathic dilatory cardiomyopathy, transplant vasculopathy after heart transplantation, homozygotic familial hypercholesterinemia, fo-cal segmental glomerulosclerosis, hemolytic-uremic syndrome, ...), intoxications, acute liver insufficiency, neo-plasmas, hyperhydration, thyreotoxicosis, etc., are treated by apheresis methods (cf. Pschyrembel (257th Edition), keyword "Plasmapherese"; www.nephrologie.de/172Apharese.htm).

Alzheimer's Disease (AD) is a progressive neurological dis-order, for which currently an effective treatment is not pos-sible. Typical of this disease are cerebral plaques which contain the amyloid β-peptide, and filamentous neuronal struc-tures from the microtubulus-associated TAU protein. Even though amyloid-β and TAU are both considered as relevant for the patho-genesis, the most recent research findings seem to suggest that amyloid-β is the major agent in the pathogenesis. Therefore, therapeutic agents are increasingly being developed which are intended to prevent the amyloid-β production, amyloid-β aggrega-tion or the neurotoxic events caused by these aggregates. A sum-mary of the therapeutic strategies for AD hitherto followed is given in the survey article by Wolfe (Nature Reviews Drug Dis-covery 1 (2002) 859-866).

Amyloid-β plaques form, starting out from the so-called amyloid-β precursor protein (APP) which is an integral transmem-brane protein (for which a physiologic function has not been clearly demonstrated either; however, the most recent research

findings suggest that the APP acts as a so-called membrane cargo receptor for kinesin I). APP is proteolytically cleaved by so-called secretases, wherein physiologically mainly an Aβ peptide having a length of 40 amino acids (Aβ_{40}) is formed. Other, shorter and longer forms of Aβ also form, primarily also a version of 42 amino acids (Aβ_{42}) which exhibits a high aggregation ability. This Aβ_{42}-form therefore also is the predominant form in amyloid plaques. The secretases (α-, and primarily β- and gamma-secretases) responsible for these different cleavages therefore also are primary targets aimed at by a possible AD treatment strategy. Therefore, it has been attempted to use modulators or inhibitors, respectively, for these enzymes in the treatment of AD (such as, e.g., benzodiazepines, sulphonamides, benzocapro-lactames).

A further gene associated with AD is apolipoprotein E, and for this there exist three allele variants (APOE2, APOE3 and APOE4). It has been shown that persons with one or two copies of APOE4 have a higher risk of AD, whereas APOE2 carriers have a lower risk, compared to the total population. It has also been shown that persons who take statins, i.e. medicaments which inhibit the cholesterol biosynthesis, have a markedly reduced risk for AD. A further strategy for the treatment of AD therefore concentrates on the inhibition of cholesterol biosynthesis, such as with statins, e.g.

A further approach for the treatment of AD relates to the inhibition of the amyloid aggregation in cerebral plaques, which i.a. also could be carried out by secretase-inhibitors. Moreover, it has also been suggested to lower the zinc content, since zinc at physiologically relevant concentrations is capable of inducing the aggregation of Aβ.

Finally, also immunological strategies have been described, e.g. an immunization with Aβ_{42}, which, however, had to be stopped due to severe side effects within the scope of a clinical study (Willke, Bild der Wissenschaft, 9 (2003), 24-28).

Further AD treatment strategies which have been suggested in the prior art relate to the prevention of APP expression and to the increase in the Aβ clearance, wherein substances that interact with the APP promoter region have been searched for for the former one. With regard to the Aβ clearance, an increase in the activity of certain proteases, such as the insulin-degrading en-

zyme and neprolysin, or the peripheral application of anti-Aβ-antibodies (De Mattos et al., PNAS 98 (15) (2001), 8850-8855) have been suggested. Finally, attempts have also been made to re-dissolve already existing amyloid plaques, e.g. by lowering the amyloid β-level in the serum of AD patients. In this context it has also been suggested to reduce the plaque deposits of β-amyloid proteins in the brain by apheresis methods (US 6,551,266, wherein the removal of macromolecules having a molecular weight of more than 500kD by apheresis is suggested), however, without this actually having been shown for AD. Yet, the dissolution by already existing plaques in brain cells directly by apheresis methods is not possible (blood/brain barrier cannot be crossed by plaques, or by molecules with >500kD).

Therefore, it has been the object of the present invention to provide a new treatment and prevention strategy for Alzheimer's Disease.

Accordingly, by the present invention an apheresis device is provided which comprises a solid carrier, which can be contacted with the blood or plasma flow and includes an amyloid-β precursor protein(APP)-binding receptor. With the present apheresis device, a purposeful clearance of APP or of APP degradation products, in particular of $A\beta_{40}$ or $A\beta_{42}$, can be carried out by means of apheresis in AD patients, or in patients who have a risk of AD. It has been known that there exists a dynamic equilibrium of $A\beta_{42}$ between the central nervous system (CNS) and the plasma. In the mouse model it could be demonstrated (DeMattos PNAS 2001, cf. above) that the peripheral application of anti-Aβ-antibodies has an influence on the CNS and plasma $A\beta_{42}$ clearance and reduces the $A\beta_{42}$ load in the brain, without the anti-Aβ-antibodies overcoming the blood/brain barrier. These results have been confirmed by Matsuoka et al. (Journal of Neuroscience 2003: 29-33) by the peripheral application of other $A\beta_{42}$-binding molecules (gelsolin and G_{M1}). The process of the formation of the plaques in the brain thus can be prevented by trapping $A\beta_{42}$ in blood. In doing so, it is not critical whether the receptors in the apheresis device which are contacted with the patient's blood or plasma are specific for $A\beta_{42}$ or for other degradation forms of APP; it is only essential that APP and its (proteolytic) degradation products, in particular $A\beta_{42}$, are eliminated from the blood by this specific adsorption, so that a "wrong" protein

degradation (i.e. to $A\beta_{42}$) will not occur. Thus, the present invention is based on an approach to an application of the apheresis that is entirely different from that of US 6,551,266, i.e. it is based on the elimination of the potential plaque building blocks, and not of the plaques as such. Besides, the elimination of plaques by means of apheresis must be dismissed as an option right from the start as not effective for the treatment of AD, since the blood apheresis is not even able to reach the regions of plaque formation in the brain.

On the other hand, the apheresis according to the invention has the decisive advantage over methods which cause a depletion of $A\beta$ within the body itself (such as e.g., in DeMattos et al., PNAS 98(15) (2001), 8850-8855 with peripheral anti-$A\beta$ antibodies), that in the present case no autoimmune responses can be triggered. Moreover, according to the invention, no substances need to be supplied to the patient which are able of acting only in the body proper (possibly only after they have been transported to a certain site), but the pathogenic agent is selectively removed, i.e., the cause of the disease is specifically extracorporeally separated, without having to eliminate the reaction products in the body.

In doing so, according to the invention the existent and already known apheresis devices in all of their embodiments can easily be adapted to the present invention. In particular, when choosing the solid carrier (and the apheresis device), the medical-technical usefulness thereof should be considered. Such carriers, methods or devices have been described i.a. in US 5,476,715, US 6,036,614, US 5,817,528 or US 6,551,266. Corresponding commercial apheresis apparatuses i.a. are also distributed by the companies Fresenius, Affina, Plasmaselect, ASAHI, Kaneka, Braun, etc., such as, e.g., the LDL-Therasorb®, the Immunosorba®, the Prosorba®, the Globaffin®, the Ig-Therasorb®, the Immusorba®, the Liposorba®, the HELP®, the DALI®, the bilirubin-bile acid absorber BR-350, the Prometheus® detoxication, the MARS®, the ADAsorb-System from Medicap or the Plasma FLO-System. All these systems - even though in their commercial form not primarily always aimed at the specific elimination of a single protein - may be adapted to the present invention without any problems by a person skilled in apheresis, e.g. as immunapheresis and/or by installing the inventive solid carrier (e.g.

as a column) in the apheresis device.

Therefore, according to the invention, by "APP binding receptors" also all those substances are to be understood, which have an affinity to the ligand APP and its biological by-products, in particular $A\beta_{42}$, and which are capable of removing these polypeptides from the blood or plasma of AD patients or of persons having an AD risk. These APP- or $A\beta_{42}$-receptors, respectively, preferably may be (polyclonal or monoclonal) antibodies, proteins, peptides, gangliosides or nucleic acids.

Particularly preferred in this respect are anti-APP antibodies, anti-$A\beta_{40}$-antibodies or anti-$A\beta_{42}$-antibodies, APP-binding proteins, in particular gelsolin, apoJ or apoE, APP-binding peptides, APP-binding gangliosides, in particular G_{M1}, or APP-binding nucleic acids, in particular aptamers, or mixtures of these receptors.

Examples of such antibodies are 3D6 ($A\beta_{1-5}$), 2H3 ($A\beta_{1-12}$), 2G3 ($A\beta_{33-40}$), 21F12 ($A\beta_{33-42}$), 12H7 ($A\beta_{33-42}$) (Johnson-Wood et al., PNAS 1997:1550-1555), 10D5, 16C11 (Bard et al., Nature Medicine 2000:916-919), the antibodies described by DeMattos et al. (2001) (m266, m243) as well as antibodies of the same specificity. Such antibodies are obtained e.g. during the immunization of mammals with vaccine formulations containing APP, $A\beta_{42}$ or fragments or variants thereof, optionally followed by cell fusion and clone selection protocols (in case of monoclonal antibodies).

Gelsolin (Matsuoka et al. 2003, see above), apoJ and apoE (DeMattos et al., 2001, see above) are further examples of APP-binding protein receptors. G_{M1} is an example of an APP-binding ganglioside receptor (Matsuoka et al., 2003, see above).

Peptides as APP-binding receptors may be assembled of D- or L-amino acids or combinations of D and L-amino acids, and may optionally have been altered by further modifications, ring closures or derivatizations. Suitable peptide receptors for $A\beta_{42}$, e.g., may be provided from peptide libraries which are commercially available. Preferably, these peptides have a length of at least 5, preferably 6, amino acids, in particular at least 8 amino acids, preferred lengths being up to 11, preferably up to 14 or 20 amino acids. According to the invention, however, also longer peptides may be used without any problems as APP-binding receptors. Furthermore, oligomers (such as, e.g., polyethylene-

imine and polylysine) are suitable as receptors.

For producing such APP-binding receptors, of course, also
phage libraries, peptide libraries (see above) or structure lib-
raries, e.g. produced by combinatorial chemistry or by means of
high throughput screening techniques for the most varying struc-
tures, are suitable.

Furthermore, also APP-binding receptors based on nucleic
acids ("aptamers"; yet also "decoy"-oligodeoxynucleotides (ds
oligonucleotides which, on account of their sequence, constitute
binding sites for transcription factors) can be employed,
wherein also the latter can be found with the most varying (oli-
gonucleotide-) libraries (eg. with 2-180 nucleic acid residues)
(e.g. Burgstaller et al., Curr. Opin. Drug Discov. Dev. 5 (5)
(2002), 690-700; Famulok et al., Acc. Chem. Res. 33 (2000), 591-
599; Mayer et al., PNAS 98 (2001), 4961-4965, and many others).
The nucleic acid backbone may, e.g, be formed by the natural
phosphorodiester compounds, but also by phosphorothioates or
combinations to or chemical variations (e.g. as PNA), wherein, ac-
cording to the invention, as the bases primarily U, T, A, C, G,
H and mC may be used. The 2'-residues of the nucleotides which
can be used according to the present invention, preferably are
H, OH, or other protective groups and modifications at the 2'-
position, it also being possible for the nucleic acids to be
otherwise modified, i.e. provided with protective groups, e.g.,
such as commonly employed in oligonucleotide synthesis. Here, by
"protective group" an esterization of the oxygen atom is to be
understood, whereas in case of 2'-modification, the -OH-group is
substituted by something else. The prior art offers numerous
possibilites for both variants, particularly preferred protect-
ive groups being methyl, allyl, propyl and the like protective
groups (i.e., e.g. 2'-OCH$_3$, 2'-O-CH=CH$_2$, etc.); particularly pre-
ferred modifications are 2'-desoxy, 2'-amino, 2'-fluoro, 2'-
bromo, 2'-azido, and metals, e.g. selenium, etc. Furthermore,
according to the invention also oligonucleotide stabilizing
techniques developed for antisense technology (Ribozyme, RNAi,
etc.) can be used for providing the nucleic acids (cf., e.g.,
the companies leading in this field ISIS, and Ribozyme Pharma-
ceuticals, in particular their patent documents and homepages).

APP-binding aptamers (which, according to the invention as
defined above, also include Aβ_{42}-binding aptamers) therefore are

also preferred APP-binding receptors within the scope of the present invention.

According to the invention, therefore, the APP-binding receptors which, preferably, are comprised of peptides, antibodies or nucleic acids, are used on a suitable carrier material for the extra-corporeal elimination of APP and its proteolytic degradation products in Alzheimer (risk) patients.

When using the present invention in the medical routine practice, it is necessary that the carrier is sterile and pyrogen-free, so that any carrier substance, or any receptor/carrier combination which meets these requirements is preferred according to the invention (cf. e.g. US 6,030,614 or US 5,476,715). Among the suitable examples are porous homopolymers, co- or ter-Polymers of vinyl-containing monomers (e.g. acrylic acid, such as, e.g., TSK Toyopearl, Fractogel TSK), carriers with modifications (activations) with oxirane-containing compounds (e.g. epichlorohydrin) and, optionally, further reactions with NH_3, amino- or carboxyl-containing compounds, or CNBr or CNCl adsorbing agents, as described in EP 110 409 A and DE 36 17 672 A. Particularly preferred adsorption materials for therapeutic purposes are suitable to prevent a loss of blood cells, do not activate the complement system or do so only slightly, and prevent aggregate formation in the extracorporeal circulation as far as possible. Furthermore, the carrier materials used, preferably should be sufficiently stable relative to sterilizing procedures also when coupled to receptors, in particular relative to ethylene-oxide saturation, glutaraldehyde saturation, gamma-irradiation, vapor treatment, UV treatment, solvent treatment and/or detergent treatment, etc.. Also products based on sepharose, agarose, acryl, vinyl, dextrane, etc. may, e.g., be employed which, preferably, comprise suitable functional groups for binding of the APP-binding receptors already in their commercially available form. Further suitable carriers also include monoliths (carriers based on cross-linked glycidyl-methacrylate-co-ethylene glycol dimethacrylate-polymer).

For coupling the receptors to the suitable carriers, the chemistry known to the person skilled in the art (e.g. Bioconjugate Techniques, Greg T. Hermanson, Ed., Academic Press Inc., San Diego, CA, 1995, 785pp.) can be used.

In a further aspect, the present invention relates to the

use of the inventive device for providing a treatment or for
providing a treatment, or a treatment device, for Alzheimer's
Disease, or for preventing such a disease, by suitably preparing
the device for the treatment of the respective patient. When
carrying out the treatment, a patient is connected to the apher-
esis apparatus for a period of time sufficient to effectively
eliminate APP polypeptides, wherein the patient's blood or
plasma flow is contacted with the solid carrier that comprises
the APP-binding receptor, whereupon APP and/or the proteolytic
degradation products of APP, in particular $A\beta_{42}$, are bound. In
the course of the apheresis treatment, of course, a peripheral
or central-venous vein access or arteriovenous fistula must be
ensured, just as a sufficient anticoagulation, and also the re-
quired quantification and measurement data have to be recorded.
Moreover, in most apheresis methods, a primary separation of
plasma and blood cells will be required prior to the plasma
treatment proper. Particular persons in whom a preventive meas-
ure will be required are persons with familial affliction, eld-
erly persons (>50, >60 or >70 years) or persons with another
risk factor for AD, in particular genetic factors.

The invention will be explained in more detail by way of the
following Examples to which, of course, it is not restricted.

1. Production of the APP receptor carrying carrier
1.1 Monolithic column

A CIM® Epoxy Monolithic column (BIA Separations, SI) is
equilibrated with 0.5 M Na phosphate buffer at pH 8.0 according
to the producer's instructions, and a monoclonal antibody
against $A\beta$ peptide is activated, also according to the producer's
instructions, and coupled to the CIM column. The column is
washed several times with phosphate buffer (+ 1 M NaCl), and
epoxy groups in excess optionally are blocked.

Quality assurance is carried out by checks in the washing
and equilibrating eluate; only acids without active epoxy-groups
and without antibody leakage in the eluate are used further on
and installed in an apharesis apparatus.

1.2 Sepharose column

An agarose bulk material (Sepharose CL4B) is aseptically
filled into a sterile and pyrogen-free container, and the mater-

ial is aseptically washed, the gel material being completely
dried under vacuum between each washing step. Subsequently, the
Sepharose is vapor-sterilized for 30 minutes at 115°C in the
autoclave.

After the sterilisation, the Sepharose is taken up in a
sterile container in 60% acetone/water and activated with CNBr
and triethylamine (14 g of CNBr per 96 ml of acetone; 30 ml of
triethylamine in 66.2 ml of 87% acetone). Then an acetone/HCl
solution was added (392 ml of sterile, pyrogen-free water;
16.3 ml of 5N HCl, 408 ml of acetone). The activated Sepharose
is washed and supplied to the coupling reaction within 2 h so as
to prevent hydrolysis of activated groups.

A sterile-filtered antibody solution (m266, and m243, re-
spectively), is introduced into the reaction vessel and stirred
for at least 90 min. Finally, the reaction solution is washed
thoroughly (with isotonic phosphate buffer), until no reaction
products are detectable in the eluate, and the antibody-coupled
Sepharose is filled into sterile and depyrogenized glass columns
with glass sintering and subjected to a final quality check
(eluate analysis with regard to reaction products, heavy metals
etc.; particle analysis, pyrogenicity; sterility).

2. Animal model for the apheresis treatment of Alzheimer pa-
tients

In recent years, at the institute for diabetes "Gerhardt
Katsch" in Karlsburg, Germany, a special extracorporeal system
has been developed for experimental apheresis in freely movable
small animals. This allows for an apherises treatment to be re-
peated on one and the same animal. Moreover, the animals used
can also be employed in follow-up examinations for long-term
evaluation of the apherises therapy. The use of this experiment-
al apheresis system has been successfully demonstrated with sev-
eral rat strains. Rats with Typ-1 diabetes and collagen TypII-
induced arthritis well-tolerated repeated apheresis treatment
provided that their body weight was above 250 g.

Before the experimental apheresis therapy is started, the
animals are provided with arterial and venous catheters. In
apheresis, initially blood cells and plasma are separated by
means of plasma filters in a first step. While the blood cells
are immediately re-infused into the animal (via the venous cath-

eter), the separated plasma is guided past the adsorption means
prepared in Example 1 (wherein the ligands are separated from
the plasma by binding to the immobilized affinity peptides), be-
fore it is returned into the animal.

Claims:

1.   An apheresis device comprising a solid carrier capable of being contacted with the blood or plasma flow, characterized in that the solid carrier includes an amyloid-ß-precursor-protein(APP)-binding receptor.

2.   A device according to claim 1, characterized in that the APP-binding receptor is selected from anti-APP antibodies, anti-$A\beta_{40}$ antibodies, anti-$A\beta_{42}$ antibodies, APP-binding proteins, in particular gelsolin, apoJ or apoE, APP-binding peptides, APP-binding ganglosides, in particular GM1, or APP-binding nucleic acids, in particular aptamers, or mixtures of these receptors.

3.   A device according to claim 1 or 2, characterized in that the carrier is a sterile and pyrogen-free column.

4.   The use of a device according to any one of claims 1 to 3, for providing a treatment of Alzheimer's Disease or for prevention of the latter.

Abstract:

     There is described an apheresis device comprising a solid
carrier capable of being contacted with the blood or plasma
flow, suitable for the treatment of patients with Alzheimer's
disease.